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DATA EVALUATION REPORT IX

STUDY TYPE: Gene Mutation-Ames Salmonella microsome mutation assay

TOX CHEM NO.309BB(?) see note under SYNONYMS below

MRID NO: not given

ACC. No. 408064-08

TEST MATERIAL: CL 182,005 (97.0%)

SYNONYMS: CL 3,4-dichloro-5-isothiazolecarboxylic acid
Note: Tox. Chem. 309BB is the potassium salt of this acid.
$C_4HNO_2SCl_2$

STUDY NUMBER(S): 87-02-005

SPONSOR: American Cyanamid Company

TESTING FACILITY: American Cyanamid Company
Agricultural Research Division
P.O. Box 400
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TITLE OF REPORT: Evaluation of CL 182,005 in the Bacterial/ Microscome Mutagenicity Test

AUTHOR(S): Traul, K. A.

REPORT ISSUED: 12/15/87

CLASSIFICATION: Acceptable (negative with/without S-9 in S. typhimurium TA98, TA100, TA1535, TA1537, TA1538 and E. coli WP2 uvrA-) at doses of up to 5000 ug/plate.

CONCLUSIONS:

1. In the absence of S-9, there was toxicity in all strains of S. typhimurium at the HDT (5000 ug/plate of CL 182005) and in some cases 2500 ug/plate. No toxicity was observed for the E. coli WP2uvrA- strain either with or without S-9, or for the S. typhiumurium strains in the presence of S-9.

2. No evidence of a dose-related increase in mutation frequency at the histidine locus was observed under the conditions of this assay (both with and without rat S9 activation) in any of the test strains (Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98 or E. coli WP2 uvrA-) used. The study was conducted in replicate, with 3 plates/strain/dose level on each occasion. The positive controls elicited the appropriate responses.

3. The study and its negative findings are acceptable.

A. MATERIALS:

1. Test compound: CL 182,005, batch no. 86-58-1, 97.0% active. Described as a tan powder. The vehicle was acetone.

2. Positive control compounds: 2-Nitrofluorene (2-NF) at 20 ug/plate (without activation) for TA98 and TA1538; 9-Aminoacridine hydrochloride (9-AA) at 50 ug/plate (without activation) for TA1537; N-methyl-N-nitro-N-nitrosoguanidine (MNNG) at 10 ug/plate (without activation) for TA1535, TA100 and WP-2 uvrA-; 2-Aminoanthracene (2-AA) at 5 ug/plate (with activation) for all strains.

3. Test microorganisms: the following Salmonella typhimurium strains, all with mutations in the histidine operon: TA 1535 and TA 100 (base-pair substitution); and TA 1537, TA 1538 and TA 98 (frameshift). All these strains also have a defective lipopolysaccharide coat (making them more permeable to large molecules), and a defect in the repair of ultraviolet-induced DNA damage. TA 98 and TA 100 carry a transfer factor plasmid conferring resistance to ampicillin and also causing an increase in errror-prone DNA repair. In addition, the Escherichia coli strain WP-2 uvrA- tryp was tested.

   The Salmonella strains were originally obtained from Dr. Bruce Ames, University of California, Berkeley, CA. The Escherichia strain was obtained from Dr. B. Bridges, MRC Cell Mutation Unit, University of Sussex, Brighton, England.

   According to the text (p. 10) genotypes of the bacteria were confirmed at regular intervals, according to the methods described by Ames et al. (1975; 1983).

4. S9: "Metabolic activation was provided by S-9 fraction of liver homogenate prepared from the livers of Aroclor treated Sprague Dawley rats, according to the methods described by Ames and coworkers" (Ames, B.N., McCann, J. and Yamasaki, E. Methods for detecting carcinogens and mutagens with the

Salmonella/mammalian microsome mutagenicity test. Mut. Res. 31: 347-364, 1975; Maron, D. M. and Ames, B. N. Revised methods for the Salmonella mutagenicity test. Mut. Res. 113: 173-215, 1983). "Activity of the S-9 was determined prior to the initiation of this study using standard mutagenic materials, 2-Aminoanthracene and 7,12-Dimethylbenzanthracene. The S-9 used for these assays was lot no. R352 prepared by Microbiological Associates, Inc., Bethesda, MD. This material was stored at about -80 degrees until the day of use. Histidine deficient top agar and tryptophan deficient top agar were also used for S. typhimurium and E. coli test strains respectively.

5. Medium: Vogel-Bonner Medium E (Vogel, H. J. and Bonner, D. M. Acetylornithinase of Escherichia coli. Partial purification and some properties. J. Biol. Chem. 218: 97-106. 1956) was used in all experiments.

B. STUDY DESIGN:

1. Mutagenicity assay:

There was no rangefinding assay for cytotoxicity. Doses used (all strains) were 0, 100, 500, 1000, 2500 and 5000 ug/plate in both the presence and absence of S-9 fraction.

The S-9 cofactor mix preparation and methods for plate incorporation were essentially those described by Ames et al. (Ames, B.N., McCann, J. and Yamasaki, E. Methods for detecting carcinogens and mutagens with the Salmonella/ mammalian microsome mutagenicity test. Mut. Res. 31: 347-364, 1975; Maron, D. M. and Ames, B. N. Revised methods for the Salmonella mutagenicity test. Mut. Res. 113: 173-215, 1983).

The S-9 cofactor mix was stored at -20° C. "Each plate having metabolic activation received 0.05 ml S-9 and 0.45 ml cofactor mix. The cofactor mix and S-9 were thawed, mixed and stored refrigerated until the test material was added."

"Tubes not receiving S-9 mix received 0.5 ml of buffer (0.1M sodium phosphate, 0.008M magnesium chloride, 0.033M potassium chloride)."

"Base layer plates containing agar and minimal medium were prepared in advance. On the day of the test 0.1 ml of the test material was mixed with 0.1 ml of bacterial cells, 0.5 ml of S-9 mix or buffer. To this mix 2.0 ml of molten top agar with trace amounts of histidine, biotin and tryptophan was added and this was poured onto the base layer and allowed to solidify. Three replicates per dose point were prepared.

After about 48 hr of incubation at 37C revertant colonies were counted. Plates were counted manually or were counted using a Biotran automatic colony counter. Concurrent positive and vehicle control plates were prepared on each test day. The assay was performed twice in its entirety."

3. Evaluation:

"For each dose level in the plate incorporation assay the mean value for revertants per plate was calculated. If the mean value for a given dose point was equal to or greater than twice the concurrent vehicle control value, the result for that dose point was considered to be positive. A positive result for the assay is defined as a reproducible dose associated increase in the mean numbers of revertant colonies over at least three concentrations of the test material with at least one positive dose point. The final decision is guided by reasoned scientific judgement."

4. An unsigned Good Laboratory Practice statement is provided on p. 11 of the report, and there is an additional signed and dated statement on p. 3. There is a Quality Assurance Unit Report on p. 17-18.

C. RESULTS:

1. Mutagenicity assay:

"CL 182,005 was tested at doses of 5000, 2500, 1000, 500 and 100 ug/plate, in the presence and absence of S-9 metabolic activation, in the plate incorporation assay. Six tester strains of bacteria were used. The assay was conducted twice with concurrent negative and positive controls."

Each of the assay runs was conducted with 3 plates/exposure level.

The results of the assay are shown in appended pages 1 through 4.

D. DISCUSSION:

In the absence of S-9, there was toxicity for all strains of Salmonella typhimurium at the highest dose level of CL 182005 (5000 ug/plate), and in some cases there was also considerable toxicity at 2500 ug/plate. No toxicity was observed for the E. coli WP2 uvrA- strain either with or without S-9, or for the S. typhiumurium strains in the presence of S-9.

No evidence of a dose-related increase in mutation frequency at the histidine locus was observed under the conditions of this assay (both with and without rat S9 activation) in any of the test strains (Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98 or E. coli WP2 uvrA-) used. The study was conducted in replicate, with 3 plates/strain/dose level on each occasion. The strain specific control compounds (2NF; MNNG; and 9-AA) and the positive control compound (2-AA) to ensure the efficacy of the activation system have given significant positive responses over the corresponding negative (acetone) control values. These positive control values demonstrated the sensitivity of the assay system with or without metabolic activation.

The test material (CL 182,005) in solution was analyzed to confirm the concentrations that were used in the mutagenicity tests (i.e., the content of CL 182,005 in the 1000 and 5000 ug/ml samples was found to be 97% and 105% of the nominal concentrations, respectively; see appended page 5).

The study and its negative findings are acceptable.